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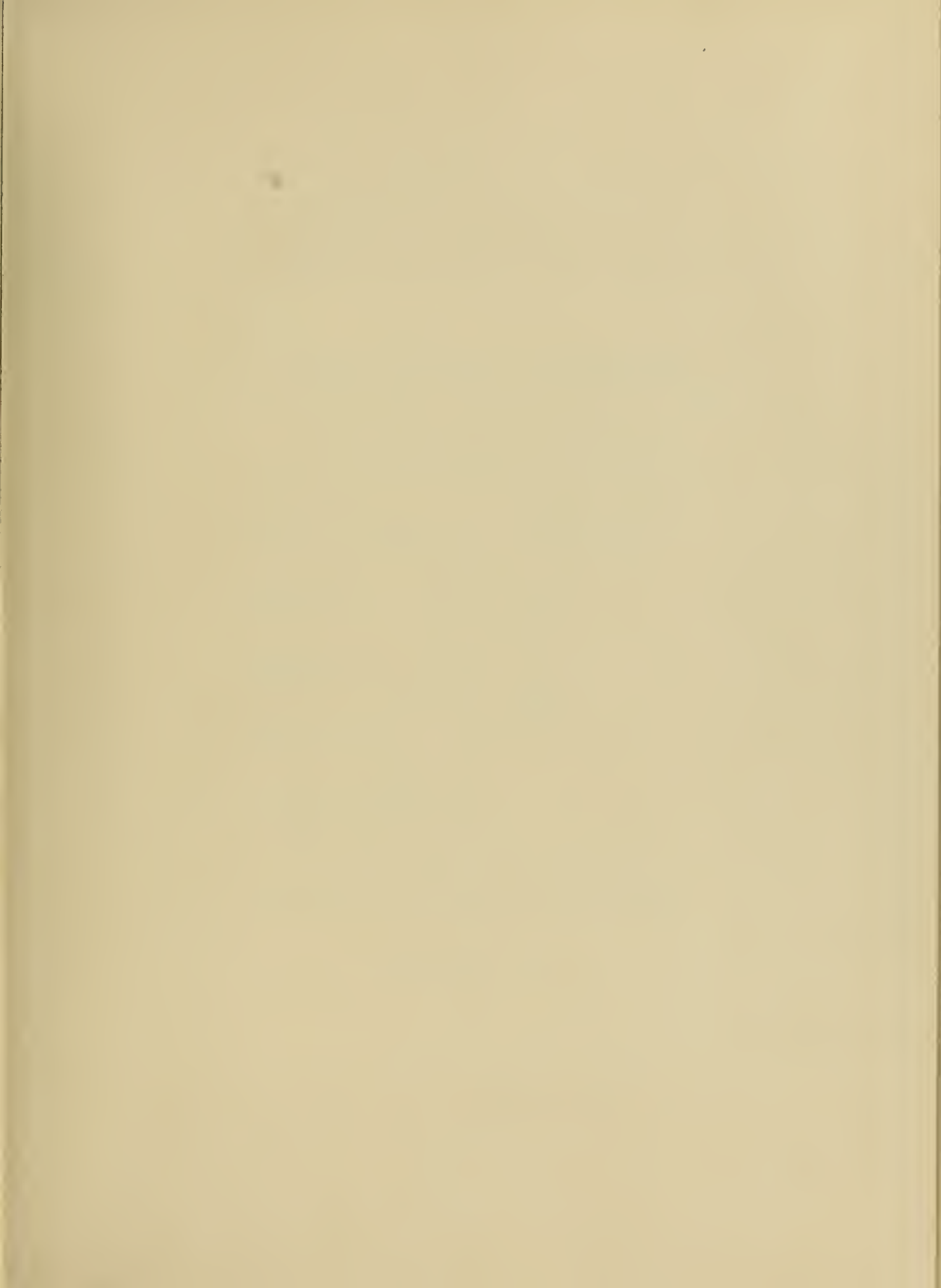
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INTERACTION BETWEEN TACTIC RESPONSES AND
METABOLIC ACTIVITIES OF MICROORGANISMS

RAYMOND L. FARMER

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1955

THESIS
F228

INTERACTION BETWEEN TACTIC RESPONSES
AND
METABOLIC ACTIVITIES OF MICROORGANISMS

by

Raymond Leroy Farmer

"

First Lieutenant, United States Army

Submitted in partial fulfillment
of the requirements
for the degree of
BACHELOR OF SCIENCE
IN
PHYSICS

United States Naval Postgraduate School
Monterey, California

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Thesis

F228

This work is accepted as fulfilling
the requirements for the degree of

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IN

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from the

United States Naval Postgraduate School

Approved:

PREFACE

The purpose of this paper was to collect and interpret as much evidence as possible pertaining to Clayton's Hypothesis, which states: In bacteria in general the ability to react to an external stimulus is necessarily linked with the active process of metabolism of nutrients at the time of stimulation, whereas protozoa have in general evolved the ability to react to an external stimulus in the absence of nutrients.

The author wishes to thank especially Professor Roderick K. Clayton for his valuable suggestions, Ahron Gibor of the Hopkins Marine Station for the privilege of working in his laboratory, and Dorothy Farmer for her assistance and patience.

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CHAPTER I

INTRODUCTION

1. The Present Problem.

The purpose of this investigation was not only to survey the literature for any kind of evidence on bacteria and protozoa concerning their responses to external stimuli, but also to correlate this information with evidence concerning metabolic changes during stimulation. It was then hoped that these combined data might help to interpret Clayton's Hypothesis: In bacteria in general the ability to react to an external stimulus is necessarily linked with the active process of metabolism of nutrients at the time of stimulation, whereas protozoa have in general evolved the ability to react to an external stimulus in the absence of nutrients.

We shall begin by reviewing some of the more important terms involved in this problem.

2. Discussion of Terms.

The central feature of all vital processes is metabolism. This term covers the intricate processes concerned in the breakdown of foodstuffs, the synthesis and degradation of cellular constituents, and the utilization of various reactions to release energy required for the proper functioning as well as for the repair and growth of protoplasm. These energy releasing reactions are of two types: respira-

tion and fermentation. Respiration utilizes the molecular oxygen of air (aerobic), whereas fermentation proceeds in the absence of air (anaerobic), and in many instances is adversely affected by the presence of oxygen.

The foodstuffs required of any organism depend primarily on the synthesizing abilities which the organism possesses. For example, a green plant is able to synthesize all its constituents from carbon dioxide, water and minerals; such an organism is known as autotrophic. In addition, a green plant requires light as a source of energy for synthesis and is therefore called photo-autotrophic. (17) Heterotrophic organisms are those which are unable to utilize carbon dioxide as the sole source of carbon but require some form of organic matter, living or dead, which has been synthesized by some other organism. (6)

Another basic feature which distinguishes living forms from nonliving matter is adaptation. Adaptation is of two sorts--the long-range adaptive modifications of evolution, and the immediate momentary adjustments to changes in the environment.

To simplify our language, we speak of a change in the environment as a stimulus. The specific change in the living system is called excitation.

The degree of excitation varies with the strength and duration of the stimulus. However, when the degree of excitation reaches a certain level, many living systems undergo

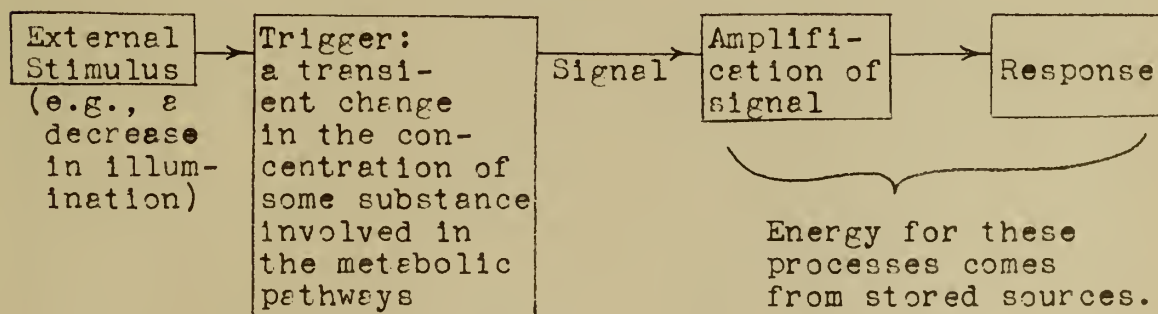


a further change which is not related in degree to the strength or duration of the stimulus, but does depend on the internal make-up of the living system. This change is called a response. In other words, when a response is elicited, its magnitude bears no necessary relation to the strength of the stimulus. Actually, a response involves the release of stored energy, and its degree is limited by the amount of stored energy available. (17)

With respect to our immediate problem, it should be pointed out that in those cases where the tactic response is definitely "linked" with an early metabolic pathway (e.g., Rhodospirillum rubrum), the connection is not that this pathway provides energy which is then diverted to the response; rather, it is that some substance is involved in the metabolism which acts as a "trigger" for the response, the energy being derived from stored sources.

Perhaps the following examples and diagram will make this clear:

Case I: Rhodospirillum rubrum



Case II: Euplena

For Euglena, the diagram is the same as above, except that the "Trigger" and its activation are incorporated in a separate mechanism which has nothing to do with the metabolism, except insofar as the latter builds and maintains the former. In other words, the trigger mechanism can operate during periods of starvation.

3. Historical Survey.

Almost unnoticed by early zoologists, enormous progress had been made in the investigation of directed movements of plants and of freely moving unicellular organisms. Here there was no danger of obscuring the real problems with anthropomorphic phrases. The scientific conception of geotropism goes back to Knight (1806) and of photo-tropism to De Candolle (1832). The conception of these two phenomena which is current today is due to Sachs (1832-87). In the ten years before 1888 a remarkable series of important publications on the movements of unicellular organisms laid the foundations of the scientific treatment of sensory physiology, and the principal new terms were invented about that time. Strasburger (1878), in a fundamental study of the movements of swarm spores and flagellates in response to light, used the term photo-taxis to distinguish the locomotory reactions of freely moving organisms from the photo-tropic reactions of sedentary plants. Then Pfeffer (1883, 1884, 1888), in an equally fundamental series of investigations on the attrac-

tion of the sperm of ferns and mosses by chemicals, established the term chemo-taxis. Schwarz (1888) described certain behavior of the green flagellates Chlamydomonas and Euglena as geo-taxis, though we know now that the term is inappropriate for these particular reactions. (5)

Engelmann (1881) was probably the first to call attention to the type of phenomenon called chemotropism. He found that certain bacteria and Infusorians gather around a source of oxygen. This was evidently a response to changes in the concentration of the oxygen, the organisms accumulating where tension of oxygen was maximum. (10)

Engelmann also made another important discovery: the photo-kinesis of Amoeba (1879), diatoms and Paramecium (1882), and particularly of Bacterium photometricum (1883). His work led to that of Jennings on the ciliates. Thermotropism was discovered by Wortmann (1883) for plants, and Verworn (1889) used the same term for reactions of Amoeba. (5)

The best summary of the whole problem of tropisms thus far is that of Jacques Loeb.

The concept of a tropism or forced movement, in the sense used by Loeb, was as follows: Animals are provided with symmetrically arranged receptors and with similarly arranged effectors. The action of a directed stimulus (e.g., a beam of light) will, if it falls unequally on the various receptors, result in unequal action by the effectors. The animal will consequently move along a curved path until such time



as the receptors are equally stimulated by the stimulus. At such time the effectors will act equally, and the animal will then move in a line determined by the direction of the stimulus. Attempts to prove that all animal reactions follow this pattern have not been successful, and the term tropism is now used mainly in its earlier and more limited sense to refer to bending responses of sessile plants and animals. The term taxis is now in general use for reactions involving locomotion. (17)

The green flagellates show a directed reaction in Loeb's sense. When they encounter a region of increased light intensity, the photosensitive eye spot in some manner causes the flagellar beat to alter so as to place the long axis of the animal in line with the light beam.

In the case of most Protozoa, however, locomotion is undirected. The classical example of this kind of behavior is provided by Paramecium. If this animal swims into a hot region, it backs and turns and starts off again in a different direction; if it again reaches the hot region, the backing and turning are repeated and so on until it can swim forward freely. Identical reactions are shown when Paramecium swims into solutions of certain chemicals. This is what Jennings called 'trial and error' and Holmes 'the selection of random movements'. (5)

Ever since 1883 (Engelmann) the so called purple bacteria have been suspected of having photochemical metabolism

similar to that of green plants. But it has only been within the past 15 years or so that some definite knowledge of their metabolism and nutrition has been obtained. (15)

Likewise with tactic responses, only since Manten (3)(12) in 1948 proposed that the phototaxis of the purple bacterium Rhodospirillum rubrum is dependent upon a sudden reduction in the rate of its photosynthetic metabolism, has new knowledge of their mechanisms been obtained.

Clayton's Hypothesis suggests that the relation between taxis and metabolism displayed by R. rubrum is found in all bacteria which exhibit tactic responses; an examination of observations recorded in the literature might elucidate this question.



CHAPTER II

BACTERIA

1. Introduction.

To better understand bacteria, their metabolic processes, and their specific reactions to stimuli, a survey of some of the more important aspects which relate to this problem are presented in this chapter.

2. Metabolism and Nutrition.

Nutrition of bacteria provides foodstuffs filling two needs: materials for the construction of protoplasm and materials serving as energy reservoirs for the endergonic physical and chemical processes of the organisms. Often it is difficult to separate these two processes and to study them separately, for the same substance absorbed by the organism may be used in part for building material and in part for fuel. (6)

When a suitable material is added to a suspension of washed aerobic bacteria, it is metabolized, oxygen is consumed, and carbon dioxide is evolved. If no assimilable nitrogen is included in the medium, multiplication does not occur and yet there may be a gain in the dry weight of the organisms. To this phenomenon is applied the term oxidative assimilation. (9)

We find that oxidative processes are required for all

anabolic syntheses. The phenomenon of oxidative assimilation is an example of how oxidation of a substrate may serve a dual purpose as a source both of energy and of intermediate metabolites required for synthetic processes. A further finding that demonstrates the importance of the assimilatory function of oxidation, in contrast to the purely energy yielding function, is the observation that oxidizable substrates of similar carbon content but dissimilar energy content can supply equal quantities of assimilated carbon during oxidative assimilation. The assimilation is at least to a certain extent more dependent on the chemical nature than on the free energy content of the oxidized substrate. (9)

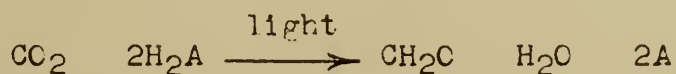
The biological conversion of carbon dioxide into organic matter in the presence of light is known as photosynthesis. Therefore, since the green and the purple bacteria are capable of producing this reaction, they are also considered to be photosynthetic organisms.

The photosynthetic bacteria are generally divided into two groups on the basis of their nutritional requirements: 1) the Thiorhodaceae, comprising the green and purple sulfur that develop in a mineral medium in the presence of hydrogen sulfide and 2) the Athiorhodaceae, embracing the purple bacteria that require organic substances for growth. (18)

The most important contributions on the photosynthetic activities of these photosynthetic bacteria have been reported by van Niel (1935, 1936 a). (20)(21)

It may be concluded from van Niel's experimental results that photosynthesis in these bacteria (often inhibited by the presence of free oxygen) requires unusual hydrogen donors for the photochemical reduction of carbon dioxide.

The general reaction:



may be used to express the metabolism of both groups of organisms. (18) Here "H₂A" is the hydrogen donor: H₂S, any of the simple organic acids, etc.

It has long been known that there exist microorganisms capable of manufacturing all their cell constituents in darkness from carbon dioxide as the only carbon source. These "chemosynthetic" or "chemoautotrophic" bacteria meet the energy requirements for carbon dioxide assimilation by the oxidation of inorganic substances. Outstanding examples are the bacteria that oxidize hydrogen, ammonia, nitrite, or reduced sulfur compounds. Studies of such bacteria have shown that chemical energy derived from the oxidation of many molecules of the inorganic substrates can actually be accumulated and used for the assimilation of a very much smaller number of carbon dioxide molecules. Hereby is revealed a definite similarity with the photosynthetic process which also requires the pooling of small amounts of energy. (22)

These chemoautotrophic bacteria accomplish a transformation of carbon dioxide into organic matter--and do so

without the benefit of radiant energy. The demonstrable existence in microorganisms of mechanisms for carbon dioxide assimilation that can function through a series of exclusively dark reactions consequently makes it possible to view with greater confidence the previously developed conclusion that in photosynthesis, too, the reduction of carbon dioxide proper must be brought about by a sequence of non-photochemical events.

In algae, as well as in purple bacteria, the occurrence of both modes of carbon dioxide assimilation has been established. Gaffron, in 1939, succeeded in so modifying the metabolic activities of a culture of Schenedesmus, by anaerobic incubation in the dark, that, upon illumination, the organisms no longer produced oxygen but carried out a photosynthetic reduction of carbon dioxide with the aid of molecular hydrogen. Their photosynthetic metabolism had thus become essentially similar to that of purple bacteria. And algae so treated can also assimilate carbon dioxide in complete darkness while oxidizing molecular hydrogen with oxygen as the final acceptor.

Similarly, those purple bacteria that can photosynthesize and grow in the presence of air, may also be grown in darkness provided air is present.

The composite of the above-mentioned facts demonstrates not only that various chemoautotrophic microorganisms are endowed with a mechanism for reducing carbon dioxide by means

of exclusively dark reactions, but furthermore that such mechanisms are encountered in normally photosynthetic organisms, and also that carbon dioxide assimilation during photosynthesis consists of a series of non-photochemical reactions. (22)

3. Early Studies in Behavior.

One of the factors most commonly determining directions of movement of bacteria is oxygen concentration. Bacteria differ greatly as to the concentration of oxygen in which they grow best, and certain motile forms very definitely seek a characteristic concentration. (1)

Engelmann (1882) made practical use of this phenomenon of aerotaxis in studying the effect of various rays of the spectrum as to their ability to induce photosynthesis in algal filaments. These latter were placed in a drop of water containing organisms which show positive aerotaxis, and a spectrum was thrown on a filament so that successive segments were in different colors. Under the microscope Engelmann could determine from which portions of the filament oxygen was evolved most rapidly (showing thereby most rapid photosynthesis) by the abundance of the bacteria clustered about the segment. As a result he was able to show that photosynthesis was most active in the red portion of the spectrum. (1)

The purple bacteria are sensitive in different degrees

to lights of different colors, tending to gather in certain colors more than in others. This was shown in a most striking way when a spectrum is thrown on a preparation of Chromatium photometricum. The largest number of the bacteria collect in the infra-red rays. There is another collection in the yellow-orange region, while a few are scattered through the green and blue. None are found in the red, the violet, or ultra-violet. (7)

Jennings (1906) wrote that it was a most interesting fact that the colors in which the bacteria collect are exactly those which are most absorbed by them, and are also those which are most favorable to their metabolic processes. This is the more remarkable when we consider that under natural conditions the bacteria rarely if ever have opportunity to react to the separated spectral colors.

Besides the purple bacteria, a green form, Bacterium chlorinum, is known to assimilate carbon dioxide and to collect in light, in the same manner as do the purple species. (7)

The movements of the organism are undoubtedly the expression of energy derived from metabolism. The organism continually takes in energy with its food and in other ways, and continually gives off this energy in activities of various sorts. The point of importance is that this activity often depends more largely on the past external conditions through which the energy was stored up than upon present ones.



Thus the organism may move without the present action of anything that may be pointed out as a specific external stimulus to this movement. It is only the change in the movement, elicited by a stimulus, for which that stimulus must account. This fact is of great importance for understanding behavior.

4. Summary.

Among different kinds of bacteria there are certain constant differences in their reactions. A relation of great significance becomes evident on examining the facts; behavior under stimulation depends on the nature of the normal life processes--especially the metabolic processes. Bacteria that require oxygen in their metabolism collect in water containing oxygen; bacteria to which oxygen is useless or harmful avoid oxygen. Bacteria that require light for the proper performance of their metabolic processes gather in light, while others do not. When one color is more favorable than others to the metabolic processes the bacteria gather in that color, even though they may under natural conditions have no experience with separated spectral colors. Keeping in mind that all these collections are formed through the fact that the organisms reverse their movement when passing out of the favorable conditions, these relations can be summed up as follows: Behavior that results in interference with the normal metabolic processes is changed, the movement being reversed, while behavior that does not result in in-



terference or that favors the metabolic processes is continued. (7)

CHAPTER III

PROTOZOA

1. Introduction.

There are excellent reasons to believe that the protozoa stand at the base of the evolutionary tree for both plant and animal kingdoms. The green flagellates cannot be separated taxonomically from the flagellated green algae. The flagellates probably arose from bacteria, among which flagellated forms are common. (17)

2. Nutritive Functions.

It is possible to set up a nutritive classification of organisms on the basis of 1) the source of energy utilized, and 2) the specific requirements for preformed compounds, especially nitrogenous compounds.

Certain of the green flagellates are normally typical photoautotrophic organisms, but most of these are facultative, that is, they can utilize certain organic compounds when these are available. Thus most of the flagellates (listed in Appendix I) can utilize acetate, many can utilize butyrate, and a few can utilize any of the organic acids from acetate (CH_3COO^-) to laurate ($\text{C}_{10}\text{H}_{21}\text{COO}^-$). Likewise, many of the green flagellates will utilize amino acids if these are supplied, although they are able to grow with nitrate or ammonia as the sole nitrogen source.



There are, however, many green flagellates which cannot subsist on nitrate, as the sole source of nitrogen. It should be pointed out that these nitrogen requirements, and probably other requirements as well, often depend on cultural conditions. Polytoma uvella, a colorless flagellate, can utilize ammonia only if ferric iron is present. Several flagellates earlier thought to require peptones can be grown on ammonium and acetate if thiamin (vitamin B₁) is supplied.

(17)

Nearly all heterotrophic organisms require thiamin for growth and activity. This compound is an essential component of an intracellular enzyme system (carboxylase) concerned with the breakdown of pyruvic acid, and hence is of primary importance in intermediary metabolism. Many flagellate protozoans, however, can synthesize thiamin.

The over all picture is one of increasing variety in nutritive requirements. For this, two alternative explanations might be proposed: 1) An increased complexity in metabolic processes, making necessary a greater variety of compounds to meet new needs; or 2) a decreased synthetic ability, making the animal more and more dependent on external supplies of compounds synthesized by more primitive organisms.

There is good reason to believe that the second explanation is the correct one. Some of the compounds required as nutrients by many protozoans--for instance, the amino acids,

thiamin, hematin--are known to be of fundamental importance in metabolic exchanges in all organisms. They are undoubtedly synthesized by photoautotrophic forms; however, there is little doubt that heterotrophic forms have lost this synthetic power. In certain cases, as with thiamin, we find organisms which can synthesize portions of the molecule if precursors are available. This fact also suggests, not a progressive increase in synthetic ability, but rather a progressive decrease. (11)

3. Feeding.

Feeding mechanisms in protozoa, as in all animals, are related to the nutritive habits of the animal. The autotrophic and saprozoic forms have no evident special feeding mechanism; they "feed" simply by the absorption of dissolved substances from the medium. It seems probable that in many species this absorption involves special chemical mechanisms and does not depend simply on diffusion, but we have no idea of the nature of such mechanisms if they exist. (17)

An amoeba feeds upon other microorganisms, especially smaller protozoa or bacteria. When one of these is encountered by the animal in its wanderings, one or more pseudopodia wrap themselves around it; they flow together and take the object into the interior of the cell. Here it is contained in a droplet of water, forming another vacuole, into which digestive enzymes are secreted. These dissolve the

microorganism, the products of digestion are absorbed into the protoplasm and become a part of the amoeba. Thus the nutrition of the amoeba is much like that of a higher animal. (6)

4. Locomotion.

The importance of locomotion in general may also be noted. Perhaps its primitive significance is in relation to diffusion. The oxygen, carbon dioxide, minerals, or dissolved foodstuffs required by small organisms may be supplied by diffusion as rapidly as they are removed by the activities of the organism, and like-wise excretory products may be removed by diffusion. If the animal reaches any great size, however, diffusion rapidly becomes inadequate, not only to bring enough materials to or remove them from the immediate vicinity of the animal, but even to allow these materials to pass into or out of the body of the animal. The rate of diffusion out of or into a cell depends upon the surface area of the cell, but the rate of metabolism is related to the total amount of living substance and hence to the volume of the cell. Since with increased size, the area increases as the square of the linear dimensions, whereas the volume increases as the cube of the same dimensions, increased size soon becomes a handicap, as the requisite rate of metabolism overtakes the diffusion capacity of the surface.



Locomotion provides a way out of this difficulty. By permitting the animal to move out of a region deficient in those materials which are being absorbed and rich in those given off, locomotion enables the animal to maintain the maximum gradient possible between the cell contents and the immediate surroundings of the cell, with respect to the various substances which must diffuse into or out of the cell. (17)

5. Early Studies in Behavior.

Change of activity is often produced by external agents. What is of interest is the fact that in any given organism the reaction to an external agent depends on the physiological condition of the organism. The nature of the reaction often depends upon whether it favors the metabolic processes; if material for these processes is lacking, the reaction to stimuli is of such a character as to secure such material. (7)

The well-fed organism reacts negatively or not at all to that stimulus to which the hungry individual reacts positively. Thus, the Paramecium bursaria that has exhausted its supply of oxygen behaves in one way with regard to light; the individual in which respiration is progressing normally, in another way. Innumerable examples illustrating this principle can be found in the behavior of lower and higher organisms. It is hardly too much to say that the progress of the metabolic and other physiological processes is the chief factor in determining the behavior of lower organisms. (7)

By way of example, the green infusorian Paramecium bursaria requires oxygen for its metabolic processes. Suppose that while swimming about, it comes to a region where oxygen is lacking. It will change its behavior, turn away, and go in some other direction. The white Paramecium caudatum also does the same, and so do many bacteria; they likewise require oxygen for their metabolic processes. All reject a region without oxygen. If, however, the water contains plenty of oxygen and the green Paramecium bursaria enter a dark region, the metabolic processes will proceed uninterruptedly. Hence, the organism does not change its behavior, but will enter the dark region without hesitation. If the oxygen in the water becomes nearly exhausted, the organism will swim about in the light and the green chlorophyll bodies which it contains produces a little oxygen which the infusorian uses in its metabolic processes. Now if the microorganism enters a dark region, the production of oxygen by the green bodies ceases; they no longer supply the metabolic processes with this necessary factor. It is then observed that the infusorian rejects the darkness and turns in another direction.

The white Paramecium caudatum does not do this, nor do the colorless bacteria. Possessing no chlorophyll, they receive no more oxygen in the light than in the darkness, and they pass into darkness as readily as into light. (7)

One of Jennings' many experiments has become a classic. A large cover-slip is supported at the corners by short legs

and the space between it and the slide is flooded with a dense suspension of Paramecium in tap-water. A bubble of carbon dioxide, a drop of soda water (i.e. carbon dioxide solution), or a drop of very dilute acetic acid solution (N/10-N/100) is then injected so as to lie under the center of the cover-slip. In less than a minute the majority of the Paramecium form a dense ring at a little distance from the acid or the bubble. As the acid diffuses outwards, the ring moves away and eventually the animals are again uniformly distributed through the fluid. Observation of single individuals shows that they are active all the time, unless they fail to turn back and thus get into too concentrated acid; normally, they turn away from the acid at some short distance from it and then turn back at some greater distance. (5)

An analogous case has been described by Fox (1921) for the flagellate, Bodo sulcatus, which reacts to oxygen gradients (and not to carbon dioxide gradients). The preferred concentration is lower than air saturation, and the flagellates themselves reduce the concentration quite quickly if they are present in sufficient numbers. The result is that they aggregate first of all in the center of the cover-slip. The resulting dense mass of flagellates reduces the oxygen concentration below the preferred value, so that they then form a ring which moves slowly outwards. At a certain distance from the center this ring becomes stabilized, and at this distance the entrance of oxygen from the air around the

edges of the cover-slip just balances the oxygen consumed by the flagellates. (5)

Behavior depends upon the metabolic processes, and is of such a character as to favor them. It is found that even when the metabolic processes of a given individual change, the behavior changes in a corresponding way.

6. Summary.

One may sum up the external factors that produce or determine reactions in Protozoa as follows: Although the organism may react to a change which is neither beneficial nor injurious, in most cases anything that tends to interfere with the normal current of life activities produces negative (avoiding) reactions, while any change that tends to restore or favor the normal life processes may produce positive reactions. Changes that in themselves neither interfere with nor assist the normal stream of life processes may produce negative or positive reactions according as they are usually followed by changes that are injurious or beneficial. Whether a given change shall produce a reaction or not, often depends on the degree to which the metabolic needs of the organism are satisfied under the existing conditions. This makes the behavior fundamentally regulatory. (7)

CHAPTER IV
CLAYTON'S HYPOTHESIS

1. General Discussion.

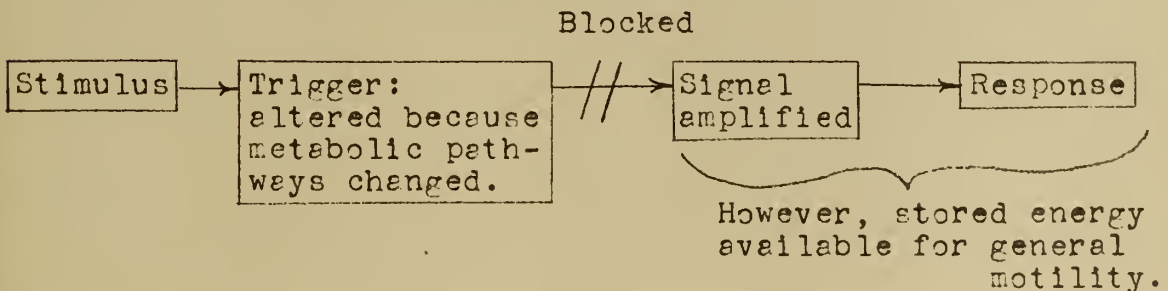
It is readily apparent among higher animals that bodily processes requiring energy do not cease during starvation. Muscular activity is possible in humans after many days without food, and the energy thus utilized is withdrawn from compounds stored within the body.

When other organisms, including bacteria and protozoa, are cut off from their food supplies metabolism usually goes on at a diminished level with the oxidation of various materials previously accumulated. This activity is called endogenous metabolism (or catabolism).

However, Clayton has speculated that there exists a difference between bacteria and protozoa in one important aspect, with respect to responses to stimulation.

As outlined briefly above (p. 3), it is hypothesized by Clayton that in bacteria there exists a "trigger" mechanism which is so intimately associated with the metabolic pathways that during starvation it cannot operate.

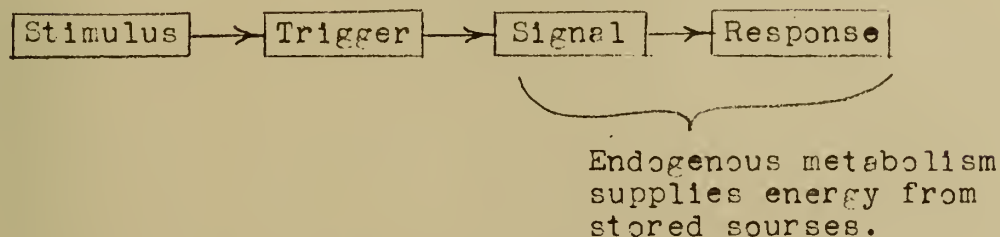
Bacterial Starvation





In protozoa on the other hand, this "trigger" mechanism can operate during periods of starvation.

Protozoan Starvation



It has been generally observed that starving animals are capable of muscular exertion, that starving protozoa respond to a stimulus, and that initially healthy motile bacteria will remain motile for a time in a properly buffered isotonic solution free of nutrients. Evidently then endogenous catabolism does yield useful energy under certain conditions.

Interesting illustrations of this later situation are the studies made of the carbon dioxide fixing capacity of the autotroph Thiobacillus thiooxidans when respiring endogenously.

Resting cell suspensions of this organism were exposed to sulfur in the absence of carbon dioxide. Phosphate uptake occurred upon the oxidation of the sulfur, but no growth was possible in the absence of a carbon source. Subsequently when the organism was freed of inorganic sulfur and carbon dioxide was introduced into the culture, carbon dioxide was fixed and phosphate simultaneously released. In this situation it is obvious that the assimilation of the carbon diox-

ide is drawing upon an endogenous substrate for energy since the exogenous energy source has been withdrawn.

When a bacterium metabolizes exogenous substrates it accumulates critical substances first and later stores reserves which often can be recognized cytologically as inclusion bodies. During starvation the reserves disappear, but the critical components are preserved until the stress becomes too great and the organism dies. Thermodynamic concepts suggest that compounds of low energy content ought to be synthesized early and be retained the longest. (9)

With these general ideas in mind further information concerning this problem was collected.

2. Bacteria.

In 1844, Draper found that, when the prismatic spectrum of the sun was thrown upon a plant, the largest amount of oxygen was liberated in the yellow-green region. And Timiriachev (1869, 1875, 1877, 1885) pointed out that utilization of light energy cannot take place unless light is absorbed by a "sensitizing" pigment. (16)

Lommel (1871, 1872) pointed out that the basic principle of photochemistry, known as Herschel's Law (no photochemical action without light absorption), requires that the spectral maximum of photosynthetic efficiency coincide with the absorption maximum of the sensitizing pigment. (16)

Timiriachev (1869, 1875), Muller (1872), Engelmann (1882)



and Reinke (1884) gave experimental proofs of this coincidence, by showing that the photosynthetic efficiency of green plants decreases steadily from red through yellow to green, parallel with the decline in absorbing capacity of chlorophyll. (16)

Engelmann (1883) noticed that the maximum of the photosynthetic efficiency of red algae lay in the green part of the spectrum, and that of blue algae, in the yellow. As in the case of the green plants, the position of the maximum of photosynthesis coincided roughly with that of the maximum of light absorption. A year later Engelmann concluded that all pigments that contribute to light absorption by the algae also contribute to photosynthesis. (16)

As we have seen previously, the reactions of freely moving organisms to light are termed phototactic movements or more briefly phototaxis, and this was described in motile animals as well as in motile plants such as green algae, flagellates, and purple bacteria.

The main objective will now be to present evidence which will indicate under what circumstances the "action spectrum" for phototaxis (sensitivity of the response vs. wave length of light) resembles that for photosynthesis (or metabolism), indicating an association between the two processes.

We owe most of our early knowledge of phototaxis in purple bacteria to the investigations of Engelmann (1888). He emphasized that the accumulation of phototactic organisms



in a spectrum which partially illuminates the microscopic field is due to the organisms' property of executing a so-called "shock reaction" (avoiding reaction) on the sudden reduction in the light intensity which the organisms undergo in entering the dark zone. As a result of this phototactic reaction, the direction of movement is reversed, which prevents the organisms from crossing the boundary from light to dark. Since, conversely, the organisms are not prevented in their random movements from entering the illuminated part out of the dark, a light zone acts like a trap. Consequently these bacteria accumulate in those parts of the spectrum which "appear brightest" to them. (14)

A most interesting fact pointed out as early as 1888 by Engelmann was that if a spectrum was thrown on the cultures of photosynthetically active bacteria, they accumulated in the absorption bands of the green pigment (which we now call bacteriochlorophyll), (16) and that the colors in which the bacteria collect are exactly those which are most absorbed by them, and are also those which are most favorable to their metabolic processes. (7)

Purple bacteria also contain numerous carotenoids, with absorption bands clearly separated from those of bacteriochlorophyll. (16) Besides the purple bacteria, a green form Bacterium chlorinum, is known to assimilate carbon dioxide and to collect in light, in the same manner as do the purple species. (7)

Turning to more recent investigations, Manten (1946), in studying the action spectrum of phototaxis of Rhodospirillum rubrum, found peaks corresponding to the absorption peaks of bacteriochlorophyll (5900 A) as well as to those of some of the carotenoids (but not the main carotenoid of these cells, spirilloxanthin). (16)

From the fact that bacteriochlorophyll is involved in phototaxis, the conclusion was drawn that phototactic reactions of purple bacteria are induced by a sudden decrease in the rate of photosynthesis. This implies that the action spectrum of phototaxis in R. rubrum is the same as the action spectrum of photosynthesis. Therefore in purple bacteria light absorbed in certain carotenoids will be active in photosynthesis. (13) However, from the comparison of the action spectrum of photosynthesis with the absorption spectra of the carotenoids, isolated by Manten, it should be concluded that the main carotenoid, spirilloxanthin, is inactive in photosynthesis. Thomas measured the action spectrum of photosynthesis and found it to be similar to Manten's action spectrum for phototaxis.

The fact that the location of the maxima and minima in both action spectra turns out to be exactly the same is strongly in favor of Manten's hypothesis concerning the identity of the action spectra of phototaxis and of photosynthesis in these bacteria. It also gives strong support to Manten's hypothesis that the phototactic reactions are based on

the photosynthetic process, and that, accordingly, the same pigments are involved in both phototaxis and photosynthesis. (19)

Duysens in 1951 showed, by studies of fluorescence, that energy is transferred between pigments in purple bacteria within the photosynthetic apparatus. The fluorescence action spectrum revealed that a partial transfer of energy from carotenoids to bacteriochlorophyll takes place, and that the action spectrum for B 890-fluorescence is proportional to the action spectrum for phototaxis (or photosynthesis). B 890 is the bacteriochlorophyll-protein distinguished by the absorption maxima of 8900 Å. (4)

In 1953 Clayton suggested that spirilloxanthin is phototactically active but that one or more of the scarcer carotenoids are much more active in proportion to their concentration in R. rubrum. He also found that the action spectrum is in good agreement with that obtained by Manten, showing the characteristic bacteriochlorophyll maxima at 5900 and 8700 Å. (2)

The most likely interpretation of the results of Manten (1948) (12), Thomas (1950) (19), Duysens (1951) (4), and Clayton (1953 I) (2) is that action spectra for phototaxis and for photosynthesis in purple bacteria are identical, with some carotenoids participating by transferring energy to bacteriochlorophyll.

Clayton (1953 II) found that the saturating light in-



tensity for phototaxis is influenced markedly by the nature of the substrate which serves for photosynthesis. This also supports Manten's hypothesis that photosynthesis and phototaxis are closely related. In particular, the effect of propionate upon the saturating intensity for phototaxis suggest that the transient change which is important in the mediation of phototaxis is localized in the region of substrate oxidation. Also described by Clayton are some observations on the aerotaxis of R. rubrum. He found that in the dark the tactic response is associated with a decrease in the rate of oxidative metabolism, just as the phototactic response is associated with a decrease in illumination which corresponds to a decrease in the rate of photosynthesis. (3)

3. Protozoa.

Some significant data concerning Protozoa were tabulated by Waterman in 1936 (23), as follows (the wave lengths most effective for phototaxis are presented):

Regions of Maximum Sensitivity, in millimicrons	Organism	Investigators
483 Blue	Euglena	Mast '17
494 Blue-Green	Volvox	Laurens, Hooker '20
524 Green	Pandorina (Flagellate)	Mast '17
525 Green	Eudorina (Flagellate)	Mast '17

We see that for all of these protozoa, light absorbed by chlorophyll is inactive for phototaxis, indicating that the tactic responses are not associated with photosynthesis. Thus, these data directly support the second half of Clayton's hypothesis.

Because of the lack of more evidence in the literature concerning protozoa, an experiment was performed by this author to test Clayton's hypothesis. This experiment follows in Chapter V.

CHAPTER V

EXPERIMENT: PHOTOTAXIS OF PLATYMONAS SUBCORDIFORMIS

1. Introduction.

The purpose of this experiment was to learn how rapidly Platymonas subcordiformis, a photosynthetic alga, reacted to light under various degrees of starvation, and to interpret the results in terms of that portion of Clayton's hypothesis which states that protozoa¹ have the ability to react to an external stimulus in the absence of exogenous metabolism.

Cells which had been exposed to light continuously were compared with cells which had been kept in the dark for five days. Both groups were tested as to their rate of phototactic response to a directed light stimulus with and without carbon dioxide available to them.

2. Experimental Method.

A method was needed by which the rate of travel of these organisms could be measured when they responded to a stimulus of light. One method which was available involved the use of calibrated centrifuge capillary tubes. This method, which involved driving all the cells to one end of a capillary tube by centrifugal force, proved to be unsuccessful. In order to drive all the algal cells into the tip of the tube,

¹The green flagellate protozoans cannot be separated taxonomically from flagellated green algae. (17)



too much force had to be employed. These organisms did not respond to any stimulus and appeared motionless under the microscope. Therefore another procedure was devised which proved very successful for this particular experiment.

In this second procedure, melting point capillary tubes were filled with sea water, and a method was devised to inject the algae into one end of the tube. All the capillary tubes used in the experiment to measure the phototactic response were of the following dimensions: length 100 mm., diameter 2 mm. The tubes were filled with sea water by capillary action.

Great care was taken not to permit any air bubbles to enter the tube, for this would interfere with the passage of algae down the tube. The tube was then sealed at one end with vaseline. (See Fig. 1)

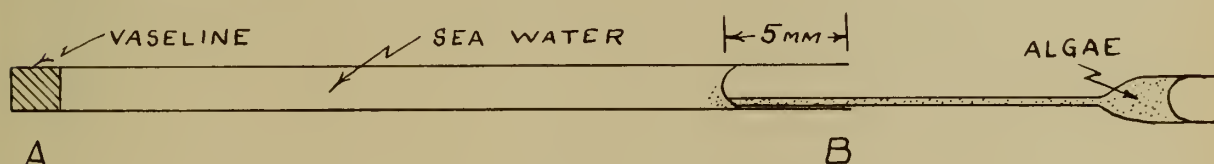


Fig. 1.
Melting Point Capillary Tube During Injection of Algae

Water was extracted from end B to a depth of 5 mm. The medium containing the cells was then placed in end B by inserting an extremely fine tube, which contained the organisms, along the interior side of the larger capillary. By this method all air bubbles were completely avoided. Hence, the remaining five millimeters of the capillary were filled with these active algae. These motile organisms were thus con-

tained in a very small portion of the capillary tube close to end B.

Again carefully avoiding air bubbles, end B was sealed.

Each end of the capillary tube was sealed with vaseline to prevent convection currents and other extraneous disturbances from molesting the algae, and to prevent the absorption of carbon dioxide into the sea water.

Every 15 minutes the capillary was placed under the microscope and the distance the algae had traveled was measured and recorded. As a workable guide, the position chosen for measurement between the zone of no cells and the zone containing cells was arbitrarily chosen to be that position to the left of which approximately ten cells existed. All other cells were to the right of this position. (See Fig. 2)

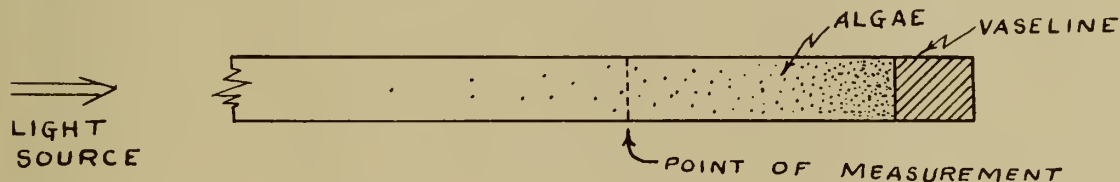


Fig. 2.
Distribution of Algae and
Position of Measurement Indicated

Eight different conditions were compared as outlined below. Three samples were taken for each condition tested.

I. "Fed" cells--those kept in a constant source of light.

1. Directed motion (phototaxis) measured.

a) Carbon dioxide available to cells

b) Carbon dioxide not available to cells

2. Random motion measured. (A control)



a) Carbon dioxide available to cells

b) Carbon dioxide not available to cells

II. "Starved" cells--those kept in the dark for five days.

1. Directed motion (phototaxis) measured.

a) Carbon dioxide available to cells

b) Carbon dioxide not available to cells

2. Random motion measured. (A control)

a) Carbon dioxide available to cells

b) Carbon dioxide not available to cells

Random motion of the cells was measured for comparison with that motion of the algae due to direct light (i.e., light from one direction only). Thus for random motion measurements, light was permitted to enter the capillary tube from all directions. Light from the windows was only permitted at right angles to the long axis of the tube.

To obtain directed motion due to light from only one direction, the capillary tubes were inserted into rubber tubing only one end of which was open. (See Fig. 3)

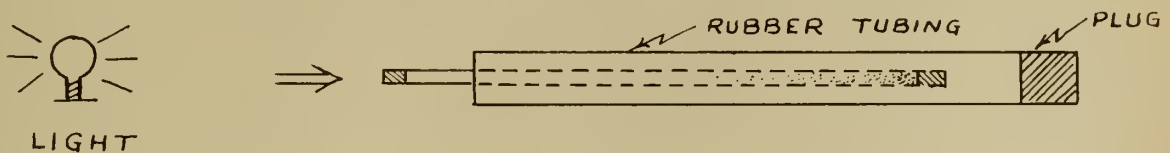


Fig. 3.
Directed Light Apparatus

The open end was pointed toward a 100 watt light source from a distance of 18 inches.

To obtain sea water free of carbon dioxide, artificial

sea water (8) was tried without success. Although it is known that these organisms will live in artificial sea water, it was found they would not respond to a directed light stimulus. In this artificial sea water all organisms appeared to cease moving and were observed to stick to the sides of the glass tube more readily than under any other condition tested.

Good positive phototactic results were obtained from carbon dioxide-free sea water in another manner. Carbon dioxide free air was vigorously passed through both the sea water and the culture medium for five minutes prior to each test in those parts of this experiment in which carbon dioxide was not desired. This carbon dioxide-free air was obtained by passing compressed air through ascarite.

The pH values compared as follows:

a) Fresh sea water: pH = 8.20

b) Carbon dioxide-free sea water: pH = 8.42

This difference in pH might possibly affect the results of this experiment. However, this effect was not investigated further.

It was surprising to note that after five days in the dark, Platymonas subcordiformis cells appeared as lively and as active as those kept in a constant light source; i.e., both test groups appeared equally energetic prior to the conducting of the experiment.



3. Results.

The results of this experiment are presented graphically in Fig. 4. (p. 39) The points plotted are averages of three runs in each case. The main features to note are:

- a) The "starved" cells reacted to the directed light stimulus more rapidly than did the well "fed" cells, both in the presence of carbon dioxide and in the absence of carbon dioxide.
- b) The cells deprived of carbon dioxide reacted more vigorously to the directed light stimulus than did those which had access to carbon dioxide.
- c) The reaction rate to a directed light stimulus was from four to eight times greater than the motion due to random movement.

4. Conclusions.

The results of this experiment indicate that starved Platymonas subcordiformis algae move more rapidly toward a light source than do well-fed algae, and that those organisms deprived of carbon dioxide are even more vigorous than those placed in natural sea water which contains carbon dioxide. Therefore, Clayton's hypothesis is confirmed in that these algae do have the ability to react to an external stimulus in the absence of exogenous metabolism.

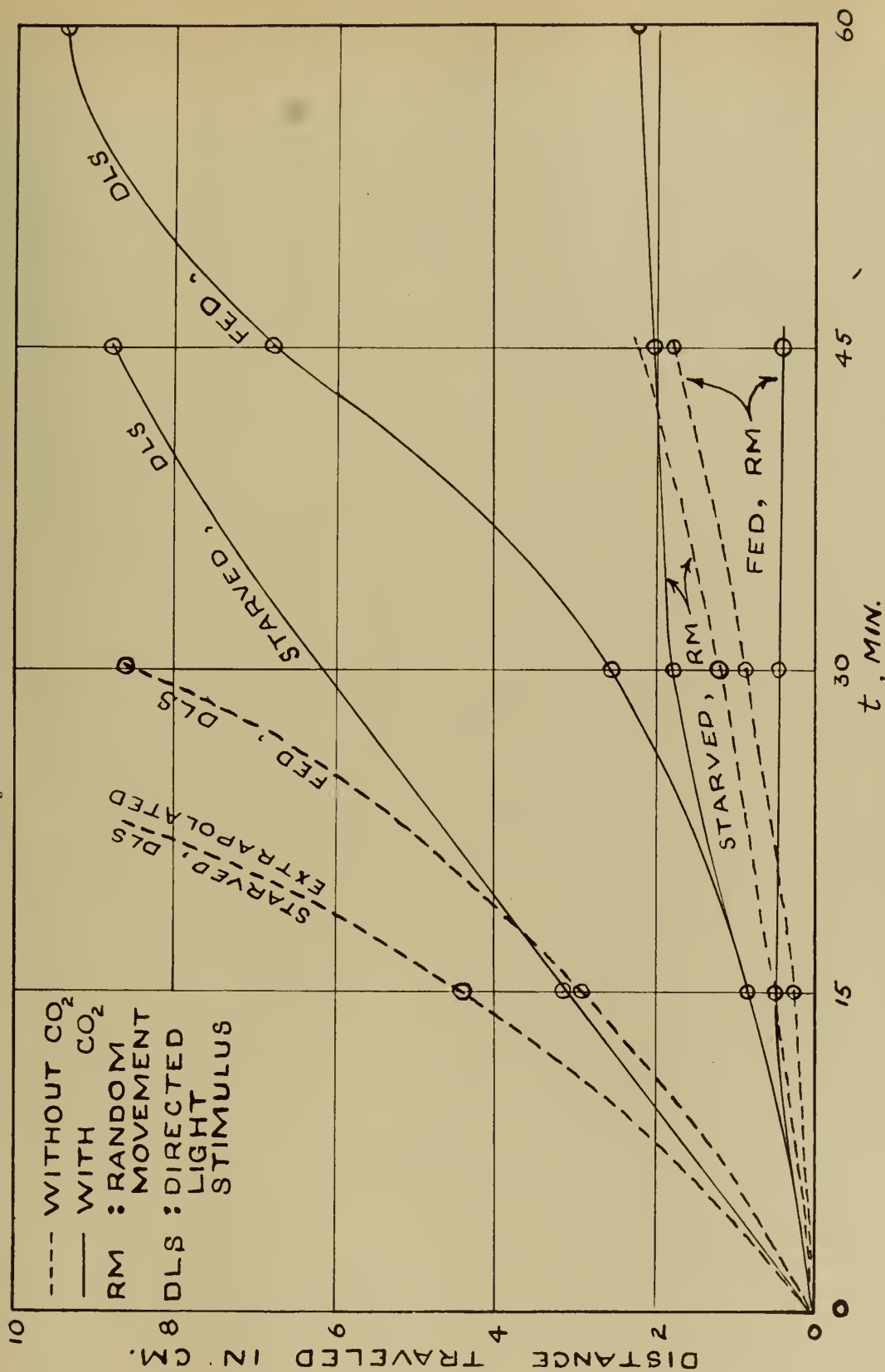


FIG 4: PHOTOTAXIS OF *PLATYMONAS SUBCORDIFORMIS*
DISTANCE TRAVELED VS. TIME UNDER VARIOUS
DEGREES OF STARVATION AND STIMULATION



CHAPTER VI

SUMMARY

After surveying the literature, initially through the Biological Abstracts and Chemical Abstracts, the information assembled above is felt to be quite complete with respect to the subject under investigation.

As we have seen, most of the evidence in the literature concerning toxic responses and metabolic activities pertain to purple bacteria. One possible reason for this is that the conditions in purple bacteria are particularly favorable for an investigation of the part played by the carotenoids in photosynthesis, since the absorption peaks of the bacterial carotenoids are not concealed behind the absorption bands of chlorophyll. (16)

The only evidence so far which bears on Clayton's hypothesis at all is in support of it. It is true, however, that we have no direct evidence pertaining to non-pigmented organisms.

This author therefore concludes that more experiments such as the one reported in Chapter V are needed before the question can be settled. In particular, one should also investigate the aerotaxis of non-pigmented protozoa and bacteria, with and without substrate, (e.g., the colorless spirilla could be used).

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APPENDIX I

UTILIZATION OF ORGANIC ACIDS BY FLAGELLATE PROTOZOANS (17)

	Utilization of Fatty Acids										
	Chlorophyll	Acetic (C ₂)	Propionic (C ₃)	Butyric (C ₄)	Iso Butyric (C ₄)	Valeric (C ₅)	Iso Valeric (C ₅)	Caproic (C ₆)	Iso Caproic (C ₆)	Heptylic (C ₇)	
Class Flagellata											
Order Phytomonadina											
Family Chlamydomonadidae											
-Chlamydomonas agloeiformis	+	+	-	+	-	-		-			
-Haematococcus pluvialis	+	+	-	+	-	-		-			
-Chlorogonium euchlorum	+	+	-	-	-	-		-			
-C. elongatum	+	+	-	+	-	-		-			
Family Polytomidae											
-Polytoma obtrusum	-	+	-	+	-	-	-	-	-	-	
-P. caudatum	-	+	-	+	-	-	-	-	-	-	
-P. uvella	-	+	-	+	-	+	-	+	+	-	
-P. ocellatum	-	+	+	+	+	+	±	+	+	+	To Lauric(C ₁₂)
-Polytomella caeca	-	+	+	+	-	+	-	+	±	+	
Order Euglenoidinia											
Family Euglenidae											
-Euglena gracilis	+	+	+	+	-	+	-	+	±	-	To Lauric(C ₁₂)
Family Astesiidae											
-Astesia quartana	-	+	+	+	±	+	±	+	+	+	
-A. chattoni	-	+	+	+	±	+	-	+	±	+	To Nonylic(C ₉)
Order Cryptomonadina											
-Chilomonas paramecium	-	+	-	+	-	+	±	+	+	-	



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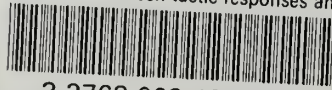
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